The Presence of Anti-Tat Antibodies Is Predictive of Long-Term Nonprogression to AIDS or Severe Immunodeficiency: Findings in a Cohort of HIV-1 Seroconverters

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The human immunodeficiency virus (HIV) type 1 Tat protein plays a key role in the life cycle of the virus and in pathogenesis and is highly conserved among HIV subtypes. On the basis of this and of safety, immunogenicity, and efficacy findings in monkeys, Tat is being tested as a vaccine in phase 1 trials. Here, we evaluated the incidence and risk of progression to advanced HIV disease by anti-Tat serostatus in a cohort of 252 HIV-1 seroconverters. The risk of progression was lower in the anti-Tat–positive subjects than in the anti-Tat–negative subjects. Progression was faster in the persistently anti-Tat–negative subjects than in the transiently anti-Tat–positive subjects, and no progression was observed in the persistently anti-Tat–positive subjects.

Tat is a regulatory protein of HIV that is expressed very early after infection and is essential for virus gene expression, replication, and transmission [1, 2]. Several studies have suggested that an immune response to Tat may play a role in the control of HIV disease progression [3–8]. The results of preclinical studies with anti-HIV Tat-based vaccines have supported this concept: vaccination has been shown to be safe and to induce specific immune responses capable of containing virus replication in monkeys and mice, and vaccination has been shown to prevent disease after pathogenic virus challenge in monkeys [9–11]. Moreover, Tat is highly conserved in its immunogenic regions among all group M subtypes [12]. Recent data have indicated cross-recognition of a clade B strain–derived (BH-10) Tat protein by serum samples from African individuals infected with different HIV-1 subtypes [12]. On the basis of these results, preventive and therapeutic phase 1 clinical trials with the native Tat protein are ongoing in Italy. In the present study, to identify immune responses that may be predictive of HIV disease progression, we evaluated the prognostic value of anti-Tat antibodies in a cohort of subjects with estimated dates of HIV-1 seroconversion both before and after introduction of highly active antiretroviral therapy (HAART).

Subjects, materials, and methods. The study population consisted of 252 individuals with estimated dates of HIV-1 seroconversion who enrolled in the Italian HIV-Seroconversion Study at 2 participating clinical centers between 1985 and 2000. Each subject had a negative HIV-1 test followed by a positive HIV-1 test within 24 months, and the date of seroconversion was estimated as the midpoint between the dates of the 2 tests [13]. For all subjects, clinical and laboratory data—including $CD4^+$ T cell counts and antiretroviral-therapy status—were collected every 6 months. Serum samples were collected after the estimated date of HIV-1 seroconversion; sequential serum samples were available for 139 (55.2%) of the 252 subjects. Viral load was determined as described elsewhere [14].

Anti-Tat IgG was detected by use of an algorithm based on 2 recently described ELISAs [12]. These validated assays were developed to obtain high levels of sensitivity and specificity using a recombinant, purified, native Tat protein derived from the IIIB strain (a BH-10 clone) of HIV-1 (clade B) as antigen [12].

The association between the presence of anti-Tat antibodies and demographic and clinical parameters of the study subjects was analyzed by use of the χ^2 test for categorical variables and the Mann-Whitney *U* test for continuous variables. The expanded AIDS case definition (category C [AIDS-defining illnesses] and category 3 [$CD4^+$ T cell count of $\leq$200 cells/μL] of the 1993 revised classification system for HIV infection) was used as the study end point. The incidence of events per 1000 person-years (p-y) and the 95% confidence intervals (CIs) were calculated for the anti-Tat–positive and anti-Tat–negative sub-

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jects. The Kaplan-Meier method was applied to estimate the cumulative probabilities of developing the end point by anti-Tat serostatus. Event-free time was defined as the interval between the estimated date of HIV-1 seroconversion and the onset of the event, death, or the end of the study (30 June 2001), whichever came first. The difference between the progression curves was assessed by use of the log-rank test. A multivariate Cox model was used to estimate the relative hazards (RHs) of reaching the end point for the anti-Tat–positive subjects and the anti-Tat–negative subjects, after adjustment for age at seroconversion, sex, exposure category, and calendar year, which was entered as a time-dependent dichotomous variable (January 1985–May 1996 vs. June 1996–June 2001). This latter variable was used as a proxy of the effect, at a population level, of HAART, which was introduced after 1 June 1996. *P* values and the 95% CIs of the RHs were calculated to assess the statistical significance of the associations. RHs were estimated with the dates of the first anti-Tat tests included as staggered entries (taking into account the different durations of infection reached at the dates). The multivariate analysis was repeated on a subset of the 139 subjects who had at least 2 consecutive anti-Tat tests; RHs were estimated for the subjects who were persistently anti-Tat positive (defined as those who had at least 2 positive tests) and for the subjects who were transiently anti-Tat positive (defined as those who had only 1 positive test).

Results. The study population consisted of 252 HIV-1–positive subjects; 166 (65.9%) were men and 86 (34.1%) were women. The median follow-up time was 7.2 years (range, 0.2–15.8 years). The median age was 28 years (range, 17–68 years). Injection drug use (IDU) was the most commonly reported exposure category (134 subjects [53.2%]), followed by heterosexual contact (63 subjects [25.0%]) and male-to-male homosexual contact (51 subjects [20.2%]).

Of the 252 subjects, 30 (11.9%) were anti-Tat positive; anti-Tat antibodies were detected at a median of 4.1 years after HIV-1 seroconversion (range, 0.1–15.0 years). There was no statistically significant difference by anti-Tat serostatus with regard to sex ($P = .257$) or exposure category ($P = .355$, for IDU vs. non-IDU); however, the anti-Tat–positive subjects were significantly younger than the anti-Tat–negative subjects (median ages, 25 vs. 29 years, respectively; $P = .042$).

Of the 252 subjects, 95 (7 of the 30 anti-Tat–positive subjects and 88 of the 220 anti-Tat–negative subjects) progressed to AIDS-defining illnesses (48 subjects) or $CD4^+$ T cell counts of $\leqslant$200 cells/μL (47 subjects), for an incidence of 59.5 events/1000 p-y (95% CI, 48.7–72.8 events/1000 p-y; calculated on the basis of 1595 p-y). Before June 1996, the proportion of subjects who had received antiretroviral monotherapy before developing AIDS was lower for the anti-Tat–positive subjects ($P = .567$, for HAART; $P = .318$, for dual therapy; and $P =$

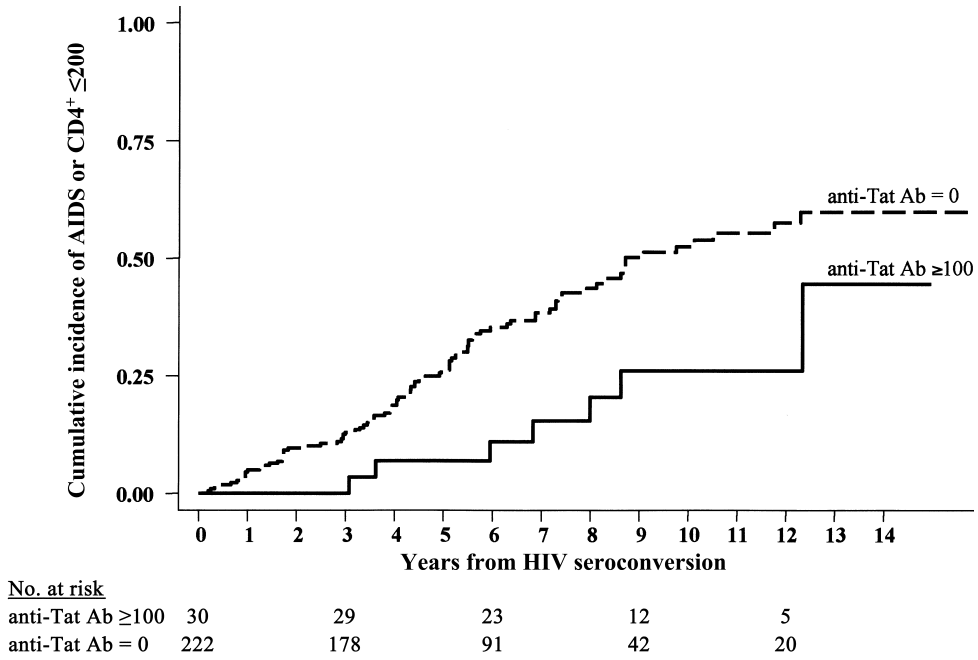

Figure 1. Kaplan-Meier curves showing progression to AIDS (cumulative incidence of AIDS or $CD4^+$ T cell counts of $\leqslant$200 cells/μL [$CD4^+ \leqslant 200$]). The cumulative incidences of AIDS or severe immunodeficiency were calculated for both the anti-Tat–positive subjects and the anti-Tat–negative subjects; disease progression was significantly slower in the positive subjects than in the negative subjects ($P = .016$, log-rank test). Anti-Tat Ab = 0, subjects negative for anti-Tat antibodies; anti-Tat Ab $\geqslant$100, subjects with anti-Tat antibody titers $\geqslant$100; no. at risk, the no. of subjects who were evaluated every 3 years, stratified by anti-Tat serostatus.

Table 1. Crude and adjusted relative hazards (RHs) of AIDS or severe immunodeficiency for anti-Tat–positive subjects vs. anti-Tat–negative subjects.

Comparison	RH (95% CI)			
	Crude	*P*	Adjusted[a]	*P*
Anti-Tat positive vs. anti-Tat negative	0.40 (0.18–0.86)	.020	0.41 (0.19–0.89)	.027
Age at seroconversion[b]	1.09 (0.86–1.38)	.478	1.13 (0.86–1.48)	.577
Sex (men vs. women)	1.20 (0.77–1.87)	.416	NI	…
Exposure category				
Man-to-man homosexual contact vs. IDU	0.83 (0.48–1.43)	.500	NI	…
Heterosexual contact vs. IDU	0.81 (0.48–1.37)	.441	NI	…
Other/unknown exposure category vs. IDU	0.00	.981	NI	…
Calendar year[c]	0.51 (0.32–0.80)	.004	0.53 (0.34–0.83)	.004

NOTE. The RHs of AIDS or severe immunodeficiency, stratified by anti-Tat serostatus, were evaluated in the 95 (of 252) subjects who reached the primary end points within the study period. CI, confidence interval; IDU, injection drug use; NI, not included in the model.

[a] Adjusted for the other variables shown in the table.

[b] RHs estimated for increments of 10 years.

[c] Follow-up after June 1996 vs. follow-up before June 1996 (used as a proxy for the effect, at a population level, of the introduction of highly active antiretroviral therapy).

.030, for monotherapy). After June 1996, a nonsignificantly higher proportion of the anti-Tat–positive subjects had received monotherapy ($P = .791$), dual therapy ($P = .093$), or HAART ($P = .932$).

The cumulative incidence of clinical or immunological AIDS events 10 years after HIV seroconversion was 26.1 events/1000 p-y (95% CI, 12.3–49.9 events/1000 p-y) in the anti-Tat–positive subjects and 53.9 events/1000 p-y (95% CI, 45.4–63.0 events/1000 p-y) in the anti-Tat–negative subjects (figure 1), and this lower incidence in the anti-Tat–positive subjects was statistically significant ($P = .016$, log-rank test). The median survival times were 12 and 9 years for the anti-Tat–positive and anti-Tat–negative subjects, respectively.

After adjustment for possible confounding variables (age at seroconversion, sex, exposure category, and calendar year), the RH of developing clinical or immunological AIDS for the anti-Tat–positive subjects versus the anti-Tat–negative subjects was 0.41 (95% CI, 0.19–0.89) (table 1). When the dates of the first anti-Tat tests were included as staggered entries in the Cox model, the adjusted RH was 0.34 (95% CI, 0.08–1.43).

The effect of HAART was also evaluated via the calendar-year analysis. For the comparison of the anti-Tat–positive subjects versus the anti-Tat–negative subjects, the RHs of developing an event (adjusted for age at seroconversion, sex, and exposure category) were 0.20 (95% CI, 0.05–0.83) and 0.66 (95% CI, 0.25–1.72) before and after the introduction of HAART, respectively.

A longitudinal analysis was performed on a subset of the 139 individuals who had at least 2 anti-Tat measurements (range, 2–8 anti-Tat measurements). Of these subjects, 119 (85.6%) were persistently anti-Tat negative, 10 (7.2%) were persistently anti-Tat positive, and 10 (7.2%) were transiently anti-Tat positive (7 who were initially positive became negative, and 3 who were initially negative became positive).

Overall, 53 (38.1%) of these 139 subjects experienced an event. None of the persistently anti-Tat–positive subjects developed an event, whereas the incidences were 28.3 events/1000 p-y (95% CI, 13.5–59.3 events/1000 p-y) in the transiently anti-Tat–positive subjects and 65.3 events/1000 p-y (95% CI, 53.0–80.5 events/1000 p-y) in the persistently anti-Tat–negative subjects. Although not statistically significant, the transiently anti-Tat–positive subjects had a nearly 70% reduction in the risk of progression, compared with the persistently anti-Tat–negative subjects (crude RH, 0.31 [95% CI, 0.07–1.27] [$P = .104$]; adjusted RH, 0.33 [95% CI, 0.08–1.38] [$P = .128$]). The adjusted RHs of clinical or immunological AIDS for the transiently anti-Tat–positive subjects versus the persistently anti-Tat–negative subjects were 0.29 (95% CI, 0.07–1.21) and 0.42 (95% CI, 0.05–3.35) before and after introduction of HAART, respectively.

We also investigated viral load patterns, applying a random coefficient model. After 1996, when viral load measurements became available, the decreases were -0.38 $\log_{10}$ copies/mL (95% CI, -0.41 to -0.18 $\log_{10}$ copies/mL) per year in the persistently anti-Tat–positive subjects and -0.27 $\log_{10}$ copies/mL (95% CI, -0.33 to -0.20 $\log_{10}$ copies/mL) per year in the persistently anti-Tat–negative and transiently anti-Tat–positive subjects combined. The difference between the slopes was not statistically significant.

Discussion. Previous studies have suggested that a humoral immune response to Tat may predict slower HIV disease progression. Cross-sectional studies have shown a higher prevalence of anti-Tat antibodies in asymptomatic HIV-infected persons than in those in advanced disease stages [3–8]. Inverse correlations between anti-Tat humoral response and both p24 antigenemia [3, 7] and plasma viral load [4] have been described. In addition, the presence of anti-Tat antibodies has been shown to be more common in nonprogressors than in fast progressors and predictive of clinical stability in nonpro-

gressors, in both cross-sectional and longitudinal studies [11, 12]. These findings suggest that anti-Tat antibodies may represent a surrogate marker of other protective factors (e.g., cytotoxic T lymphocyte [CTL] response). The above-mentioned studies, however, may have been limited by a small population size, a short duration of follow-up, or a lack of information on HIV seroconversion date.

The results of the present study reveal a strong association between the presence of anti-Tat antibodies and a slower progression to clinical and immunological (AIDS-defining) end points. After adjustment for age and calendar year (the latter as a proxy for introduction of HAART), the anti-Tat–positive subjects in our study had a 60% lower risk of disease progression, compared with the anti-Tat–negative subjects. The analysis of the subjects with sequential serum samples indicated that the persistently anti-Tat–positive subjects had the lowest risk of disease progression, whereas the persistently anti-Tat–negative subjects had the highest risk of disease progression. Furthermore, the transiently anti-Tat–positive subjects had a nearly 70% lower risk of progression, compared with the persistently anti-Tat–negative subjects. These results provide evidence that the presence of anti-Tat antibodies is a good predictor of slower progression of HIV disease.

Before firm conclusions can be drawn, however, the limitations and biases of the present study should be mentioned. First, whether anti-Tat antibodies are a proxy of other immune effector mechanisms, such as specific cellular immunity or immune response to other regulatory HIV proteins, remains unclear. Second, the anti-Tat antibody prevalence found in our study population was lower than that found in other study populations, which ranges from 13% in persons with prolonged p24 antigenemia, to 29% in recently infected persons [15], and to 100% in asymptomatic persons [3]. This phenomenon may be due to differences in the study populations (e.g., different disease stages) or to the lack of assay standardization and may affect interstudy comparisons, but it is unlikely to affect estimates of the strength of the association between antibody profile and the risk of disease progression. Third, the lag time between the estimated date of seroconversion and the date of collection of the first serum sample was greater in the anti-Tat–positive subjects than in the anti-Tat–negative subjects. However, when the dates of collection of the first serum sample were included as staggered entries in the survival analysis, the magnitude of the RH of the main end point did not change significantly. Fourth, viral load data were available since 1996 only; therefore, they should be interpreted with caution. Finally, adjustment by age was crucial with respect to dealing with confounding variables, because of the wide age range and the higher prevalence of anti-Tat antibodies in the younger subjects.

In conclusion, the presence of anti-Tat antibodies is a predictor of slower HIV disease progression. The association remains after adjustment for calendar year, considered as a proxy for the introduction of HAART. The issue of whether anti-Tat antibodies play a direct protective role or are only an indirect marker of protection merits further investigation. Nevertheless, in concert with the assessment of cell-mediated immunity (T helper cells and CTLs), anti-Tat antibodies may be a valuable tool with which to monitor the immunogenicity of Tat-based vaccines during advanced clinical testing.

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